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INSECTICIDAL AND FUNGICIDAL EFFECT OF PLANT EXTRACT-A LABORATORY BASED STUDY

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ABSTRACT

The alternatives of Bio hazardous chemical insecticide were evaluated in the laboratory with methanol, ethyl acetate and petroleum ether extract of *Lantana camara Linn.* against Sand fly (*Phlebotomus argentipes*), methanol extract against Diamond Back Moth (*Plutella xylostella)*, Red Spider Mites (*Tetranychus urticae*) and Fungi (*Pyricularia oryzae*, *Colletotrichum capsici*, *Fusarium oxysporum* -MTCC, Chandigarh) were evaluated. All were found effective against *P. argentipes* (max. 87.5% methanol extract), *P. xylostella, T. urticae* (max.100% methanol extract) and antifeedancy of *P. xylostella (*max. 83% methanol extract). The growth inhibition of *P. oryzae*, *F. oxysporum*, *C. capsici* with methanol extract was found 28.2, 22.4 and 19.2% on 9th day. The study will lead to explore the findings in broader areas like fungicidal, larvicidal and adulticidal effect to insect having medical as well as economic importance with eco-friendly plant products.

KEYWORDS: *Lantana camara*, Insecticide, Sand Fly, Diamond Back Moth, Red Spider Mite

INTRODUCTION

Lantana camara (Linnaeus) is a noxious weed belonging to Verbenaceae family which comprises of about 650 species spread over 60 countries. They are native to tropical and warm regions worldwide. They are mostly cultivated for their ornamental purpose because of their flowers which can be pink, orange, yellow, white lilac depending on the variety.

L. camara leaves have been reported to make animals ill after ingestion and its berries are toxic before they become ripe (Mc Lennan and Amos, 1989; Motion, 1994; Wolfson and Solomon, 1964). *L. camara* oil and extracts are used in herbal medicine for the treatment of various human diseases such as skin itches, leprosy, cancers, chicken pox, measles, asthma, ulcers, tumors, high blood pressure, tetanus, rheumatism, etc (Begun et al., 1995; Ghisalberti, 2000; Ross, 1999). Extracts from the leaves have been reported to have antimicrobial, fungicidal, insecticidal and nematicidal activity (Begun et al., 1995; Deena and Thoppil, 2000; Saksena and Tripathi, 1985; Sharma et al., 1999).

Bihar is endemic for Kala-azar and sharing 80 per cent of world cases taking toll of life every year since more than century. DDT (2,2-bis (p-chlorophenyl)-1,1,1-trichloroethane) is the insecticide of choice to control sand flies which is developing resistance in some part of the endemic areas of Bihar, India (Dinesh et al., 2010; Singh RK et al., 2012).

In the Philippines, the *P.xylostella* exhibited multiple resistances to malathion, methyl parathion, DDT, diazinon, mevinphos, dichlorvos, and carbaryl (Barroga and Morallo-Rejesus, 1976). Hence this study was designed to find out an alternative to chemical insecticides against various species of insects (vector and pest) and fungi in the benefit of common people with the botanicals of eco-friendly use.

MATERIALS AND METHODS

Extract Preparation

Plants were collected from endemic areas of Kala-azar of Patna districts during the flowering seasons. The dry leaves infusion was prepared following the standard technique after proper sterilization. Crude extracts in different solvents were collected (w/v, 1:10; 10g: 100ml) by soaking the infusions in different solvents like Petroleum ether, Ethyl Acetate and Methanol in different flasks providing 48 hrs incubation at room temperature. After collection and preservation of filtrates the solid parts were retreated twice separately with 100 ml of each solvent separately by following previous methods. The filtrates were crystallized at 40^0C and preserved at -20^0C for further bioassay test.

Bioassays with Adult Insects

P. argentipes

The tube bio-assay experiments were conducted in the laboratory using newly emerged laboratory bred *P. argentipes* 3 day old fed on 10% glucose solution following the WHO protocol (1998) while impregnation process was indigenous. 50µl of crude extracts of plant samples were blotted on 1.31 cm^2 area of whattmann filter paper. The filter paper was dried at 40^0C and placed in a tube. 20 sand flies were aspirated in the tube and kept overnight for bioassay. Knockdown was observed after 30 minutes and mortality was observed after 24 hrs. The same protocol was applied to negative control, control and positive control experiments in which sand flies were aspirated into tubes containing filter papers blotted with distilled water, solvent used for extraction, Deltamethrin respectively and dried in the same condition as for the extracts. Mortality was recorded at 24 hrs intervals. Where mortality in the control groups fell between 5 and 40%, the observed percentage mortality was corrected using Abbott's formula (1925).

P. xylostella

Maintenance of Pupae and Adults

The culture of Diamond Back Moth (DBM), *P. xylostella* was initiated by collecting pupae from the fields of cabbage and cauliflower. The field collected pupae were maintained in isolation to eliminate the emerging parasitoids and diseases, if any. Collected Pupae about 20 pairs were placed in petriplates kept inside a polyvinyl chamber (30 X 30 cm) to obtain adults. The moths were provided with 50% honey solution and water on two cotton swabs placed in small plastic cups. These adult females' laid eggs on the mustard seedlings kept in the polyvinyl chamber.

Once the pure culture was established, the mass production commenced from the first laboratory generation. Mustard seeds were sown obtained from the field/farm in small plastic cups (9X8 cm) containing planting medium (Vermiculate: Coco peat @ 1:3). Pots were irrigated properly at regular intervals to obtain the seedlings. The mustard seedlings exposed for egg laying were removed from the chamber and kept on a plastic tray (29X25 cm) having small quantity of water. After incubation period of 3-8 days pale green larvae emerged from the egg. 3^{rd} Instar larvae were collected for the bio-assay.

Bio-Assay

0.1% test solutions were prepared by dissolving 0.50 gm of plant extract in 0.50 gm IPA and 0.50 gm Tween 20 and make up the volume to 50 ml in D/W. The leaf discs were immersed in the test solution for 2 minutes, air dried and placed over the moist cotton wool in the petriplates. Ten 3^{rd} Instar larvae were put on these plates for feeding and the mortality was observed after 1^{st}, 3^{rd}, 6^{th}, 7^{th}, 10^{th} day after treatment. The test was done in triplicates along with Control (methanol), Blank (IPA+ Tween 20) and an untreated control (D/W). The mortality was calculated by Abbott's formula (1925).

Feeding Deterrency

A true antifeedant gives insects the opportunity to feed on the plant, but the food intake is reduced until the insects die from starvation (Wright, 1963). Antifeedancy was calculated by dry method with modifications (Morallo-Rejesus, 1985). Outline of mustard leaf was marked on a graph paper and larvae were allowed to feed on leaf treated with the test solution prepared. The % antifeedency was calculated after 2^{nd} of treatment by plotting the non-green part of the leaf on graph paper and area of feeding was calculated by counting the no. of units covered in the graph. The test was done in triplicates along with a control (methanol), blank (IPA) and untreated control (D/W). The % antifeedancy was calculated.

T. urticae

Collection and Maintenance of Culture

Mites were collected from the infested leaves in polythene bags from fields of vegetable crops. The fresh uninfected mulberry leaves were collected from the self-cultivated plants. The leaves were cut into bits (approx. 3" X 3") and placed over the moist cotton wool in petriplates (diameter 6") facing the upper surface of leaf down. The cotton wool was regularly moistened by adding water using dropper. The mites were released on it to establish pure culture. As the mite colony established, leaves were cut into four pieces and placed over four new plates (prepared as above). The leaf bits were placed in such a way that the mites could easily migrate to the freshly prepared leaf bits and the culture was maintained continuously in the laboratory and used for bio-assay when required.

Bio-Assay

The fresh uninfected mulberry leaves were collected from the self-cultivated plants. The leaves were cleaned with distilled water and they were cut into leaf discs of 14 mm diameter using a cork borer and placed bottom side up over moist strip of cotton wool (7X3cm) in Petri plates(3.5" diameter). Leaf discs were placed in each Petri plates and marked as A, B, C. These plates were scanned under binocular stereo zoom microscope to check for any live material. 0.5% of plant extracts was measured using pipette or analytical balance and dissolved with solvent (distilled water/other solvents) in a volumetric flask to prepare the solutions. A blank (IPA+ Tween 20) and untreated control (D/W) along with the test solutions were prepared. The plates were sprayed with leaf discs using glass atomizer from a distance of 30cm. Equal volume of test solution were sprayed to all the plates, air dried and ten mites per leaf disc were released on it. The mortality rate was observed at an interval of 24 hours for up to five days. The mortality was calculated by Abbott's formula (1925).

Bioassay for Fungicidal Activity

Fungal Growth Media Preparation

The readymade Potato Dextrose Agar (PDA) media (PDB @ 24 g l^{-1}) (Himedia) was weighed, dissolved in distilled water and made up to the volume. The media was dispensed in conical flasks and autoclaved at 15 psi for 20 minutes. The authentic fungal cultures from Microbial Type Culture Collection Centre, Chandigarh, were revived under aseptic conditions. Inoculum was removed from the authentic slant by means of a sterile inoculation loop and streaked onto pre-sterilized selective medium in replicates.

Inoculated plates were incubated at 25°C for 7 days *P. oryzae*, C. *capsici* and *F. oxysporum* during which fungal growth was observed and till it got sporulated. This formed the Ist filial generation or F1 generation. On obtaining confluent growth, sub-culturing was done onto vials and slants, incubated and refrigerated. Inoculum from the previous generation (F1) was used to raise subsequent generations, namely F2, F3 and F4 generations. This procedure was continued on monthly basis.

Bio-Assay

The solubility of different plant extracts in different solvents were tested before the initiation of bioassay experiment. The solvents tested were acetone, DMSO (Dimethyl sulphoxide) at concentrations 0.1 per cent. The organic solvent selected for bioassay was tested for fungal growth inhibition. The effect was tested by incorporating the organic solvent alone at varying concentrations (0.1 %), surfactant (tween 85 @ 0.1 %) alone without Plant extracts to the sterilized medium. Homogenous solution was obtained by dissolving the plant extract in appropriate solvent and surfactant. The plant extracts were tested at 0.5, 0.3, 0.1 per cent concentration.

The sterilized medium was allowed to bring down the temperature to 40-42°C and the test sample was added to medium aseptically. The medium was then poured into sterilized petriplates and allowed to solidify.

Preparation of Fungal Discs

The pure fungal culture (7 day old) of *P. oryzae, F. oxysporium, and C. capsici* were inoculated into Potato Dextrose Agar (PDA) using sterile inoculation loop in the laminar airflow. Inoculated flasks were incubated at 25°C for 7 days. After 3 days fungal disc was used for bioassay experiments. The sterile cork borer of desired diameter was unwrapped under aseptic conditions and pressed onto the surface of the plate having fungal growth to obtain neat discs. The fungal discs were placed at the center of the petridish having the plant extracts of appropriate concentration. Control/check plates were also prepared to which neither the plant extracts nor organic solvent has been added prior to inoculation. Blank plates were also prepared by incorporating the same quantity of organic solvent to the sterilized medium prior to inoculation and this formed a treatment. Plates were incubated inverted in an incubator set between 25 ± 2ºC. The observations were taken for a period of 9 days for *C. capsici* & *F. oxysporum* and *P. oryzae*. The growth of the fungus is a measure of the diameter/radial growth measured at two points, at right angles to one another. The fungal growth in terms of colony diameter was measured on specific days (3, 5, 7, and 9) from the center of the fungal disc at two points (right angles to one another). Observations in terms of fungal growth were made with the treated plates as against the control and blank plates were taken as a measure of fungi toxicity. The fungi toxicity in terms of Percentage Growth Inhibition (% GI) was calculated by means of Vincent's formula (1927).

All experiments were done in triplicate whereby mortality between 10 and 90% was considered and data entered into Microsoft Excel program. Control groups in the experimental bioassays with >20% mortality were repeated. The percentage mortality was calculated and corrected using Abbott's formula (1925).The % antifeedancy was calculated by the formulae:

$$\% \text{ Antifeedancy} = \frac{\text{Area fed in test-Area fed in control X 100}}{\text{100-Area fed in control}}$$

The fungi toxicity in terms of Percentage Growth Inhibition (% GI) was calculated by means of Vincent's formula (1927)

$$\% \text{ Growth Inhibition} = \frac{\text{(Final growth in control plates–Final growth in treated plates) X 100}}{\text{Final growth in control plates}}$$

(Treatments include the sample under evaluation, blank and control plates)

The results were expressed as mean % corrected mortality ± Standard Error. These data could be analyzed statistically.

RESULTS

Percentage yield of Petroleum ether, Ethyl acetate & Methanol extract was found to be 5.4%, 5.6% & 5.74% respectively. The corrected % mortality rate of adult *P.argentipes* after 24 hrs of exposure to crude Petroleum ether (3600 mg/L), Ethyl acetate (2800 mg/L) & Methanol (4500 mg/L) extract was found to be 39.4%,13.33 & 87.5% respectively (Figure 1) . The methanol extract of *L.camara* showed mortality rate of 81.72%, 96.77%, 100% and 16.67 %, 20 %, 53.33 % (Figure 2 & Figure 3) on day 1, day 3 and day 6 for *P.xylostella and T. urticae* respectively at 0.1 % (1000 mg/L) concentration. Percentage of antifeedancy of *P.xylostella* after 2nd day of treatment for methanol extract (1000 mg/L) was observed to be 83 ±0.017 %. The maximum growth inhibition of *P.oryzae*, *F.oxysporum*, *C.capscii* was observed at 0.5% (5000 mg/L), 0.3% (3000 mg/L), 0.3% (3000 mg/L) concentrations respectively of methanol extract showing 28.2%, 22.4%, 19.2% (Figure 4 & Figure 5) of inhibition respectively on 9th day.

DISCUSSIONS

The mortality was attributed to the antifeedant properties of the extracts (Kulkarni et al., 1997; Mehta et al., 1995). In *L. camara*, the triterpenoid lantadene was reported to be responsible for its antifeedant properties (Mehta et al., 1995). Once ingested, their effects are to prevent food utilization by susceptible insects and therefore mortality results from starvation. Phytol, a diterpene has been reported in higher concentration in the methanol leaf extract of *Lantana camara*. Lantadenes present in all *L. camara* has been reported earlier to be responsible for almost all the biological activities (Barre et al., 1997). In addition, other secondary metabolites such as alkaloids, terpenoids, and phenolics could be held partially responsible for some of these biological activities (Barre et al., 1997). 2, 3-dihydro-3, 5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) was found to be present in both the methanol leaf and flowerZ extract of *Lantana camara*. Medicinal activity DDMP was observed by Ban et al (2007).

CONCLUSIONS

We can conclude from this study that the presence of these phytochemicals in *Lantana camara* might be the reason for its insecticidal activity. The results of this experiment indicated that the shrub could be studied further in detail and its beneficial effect to the control of vector borne diseases could be utilized for healthy environments.

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APPENDICES

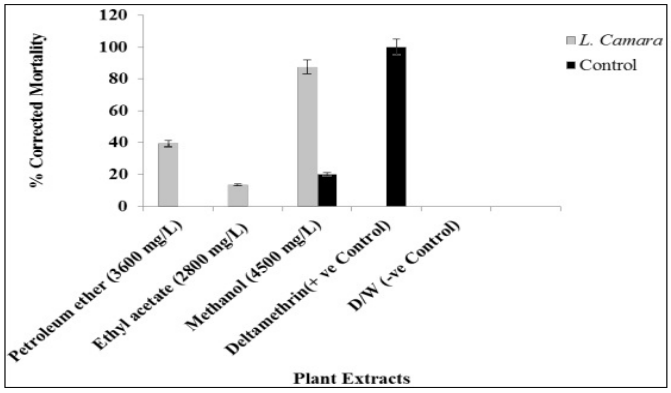


Figure 1: Mortality of *P. argentipes* against Different Extracts of *L. camara* Leaves

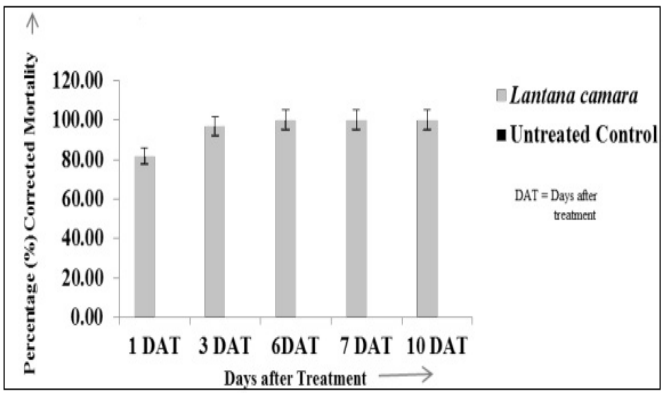


Figure 2: Mortality of *P.xylostella* at (0.1%) Concentration of Methanol Extract

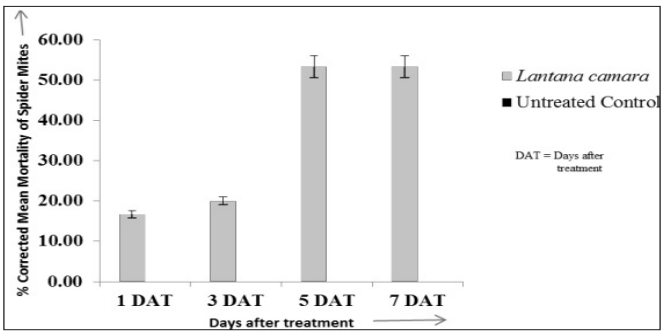


Figure 3: Mortality of *T.urticae* at (0.1%) Concentration of Methanol Extract

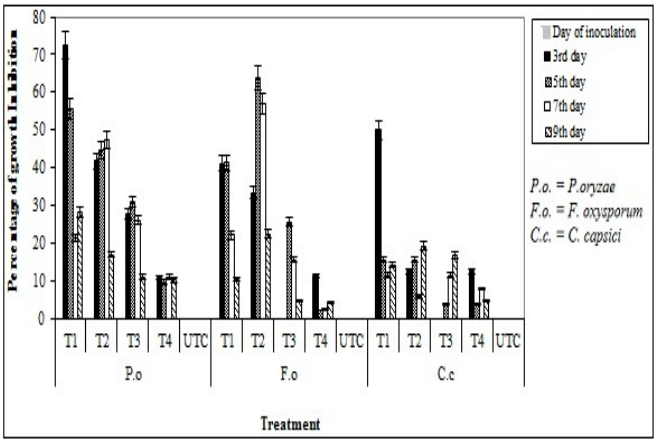


Figure 4: Percentage Growth Inhibition of Methanol Leaf Extract on Different Fungal Cultures

Where,

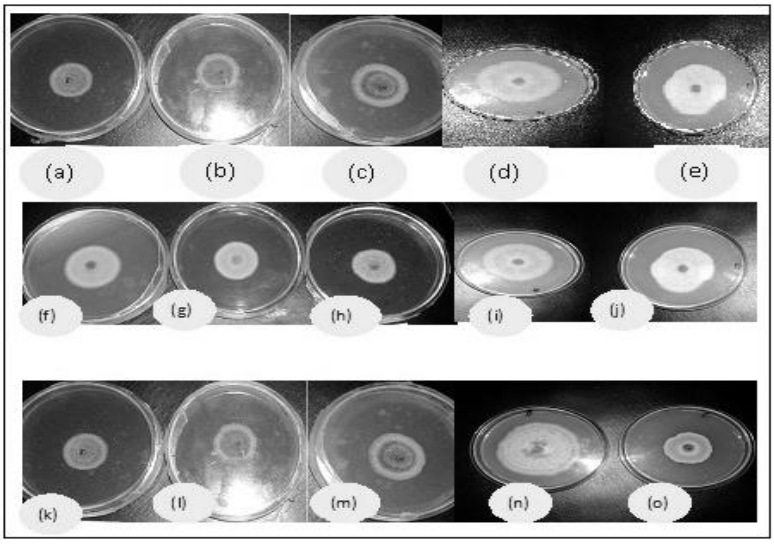
T1=0.5% *Lantana camara* extract + 0.1% Acetone + 0.1% Tween 85

T2=0.3% *Lantana camara* extract + 0.1% Acetone + 0.1% Tween 85

T3=0.1% *Lantana camara* extract + 0.1% Acetone + 0.1% Tween 85

T4=0.1% Acetone + 0.1%Tween 85 (Blank)

T5=Untreated control (UTC)



(a) T1 (0.5 %) on *P. oryzae* (b) T2 (0.3%) on *P. oryzae* (c) T3 (0.1%) on *P. oryzae*
(d) T4 (Blank) on *P. oryzae* (e) T5 (control) on *P. oryzae* (f) T1 (0.5 %) on *F. oxysporum*
(g) T2 (0.3%) on *F. oxysporum*(h) T3 (0.1%) on *F. oxysporum* (i) T4 (Blank) on *F. oxysporum*
(j) T5 (control) on *F. oxysporum* (k) T1 (0.5 %) on *C. capscii* (l) T2 (0.3%) on *C. capscii*
(m) T3 (0.1%) on *C. capscii* (n) T4 (Blank) on *C. capscii* (o) T5 (control) on *C. capscii*

Figure 5: Treatment with Different Concentrations of Methanol Extract of *Lantana camara*

Where,

T1=0.5% *Lantana camara* extract + 0.1% Acetone + 0.1% Tween 85

T2=0.3% *Lantana camara* extract + 0.1% Acetone + 0.1% Tween 85

T3=0.1% *Lantana camara* extract + 0.1% Acetone + 0.1% Tween 85

T4=0.1% Acetone + 0.1%Tween 85 (Blank)